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ASEXUAL PROPAGATION TECHNIQUES TO ENHANCE BREEDING STRATEGIES FOR LARGE SEEDED PULSE CROPS (DRYBEAN, FABA BEAN AND PEA)

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To the

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ASEXUAL PROPAGATION TECHNIQUES TO ENHANCE BREEDING STRATEGIES FOR LARGE SEEDED PULSE CROPS (DRY BEAN, FABA BEAN AND PEA)

1. Abstract / Summary Large-seeded pulse crops like common bean, faba bean and pea represent 3 of the 4 most important grain legumes for direct human consumption in the world. Pea and common bean are important economic crops in western Canada, and potential for growing faba bean is being explored. The genetic improvement effort for these crops includes introduction of genes from unadapted parents and wild species as a way to improve productivity, to transfer disease resistance, to reduce input costs and increasingly, to improve specific quality traits. The two barriers that we cannot overcome are: (1) the low number of F_1 seeds produced per pollination in large-seed legumes (1-4 seeds) compared to oilseed and cereal crops; and (2) the low seed increase ratio – a typical F_1 plant often produces only 50-100 seeds under ideal conditions. These constraints are amplified further in common bean where we consider the possibility of using wild species as gene donors. Hybrids produced through wide or intervarietal hybridizations often require screening for multiple breeding objectives. Alternative techniques are essential for timely production of large numbers of clones of hybrids in order to develop large segregating populations. This is especially important for F1 plants with genetic combinations that can be identified and selected by molecular markers. Clones produced through asexual reproduction will allow the breeder to screen for multiple traits in the F_1 generation and a large remnant population could be maintained for further evaluation. We have already successfully developed a set of techniques for lentil breeding and are now regularly using them in specific situations. Grafting, rooted cuttings and micro-propagation are the most widely used propagation techniques in many tree legumes and horticultural crops. Rooting through stem cuttings is generally not successful in pulse crops especially in faba bean, or in interspecific hybrids between common bean and *Phaseolus acutifolius* and *P. angustissimus* which are useful sources of frost tolerance and disease resistance. Our objectives were to standardize an efficient and effective alternative asexual propagation technique to increase the breeding efficiency through early generation cloning of dry bean, faba bean and pea plants. Grafting (wedge / cleft) as an efficient alternative technique was standardized in common bean using five different root stocks and five different scions including the two wild *Phaseolus* species. Stage of the root stock for grafting was standardized. Experiments with 300 grafts (30 combinations) were conducted and the best root stock for common bean across species was identified. It was estimated that more than 90% success in grafting is possible. *P. vulgaris* 'ICA Pijao', a widely used female parent in bean interspecies breeding, was found to be the best asexually compatible root stock. It had high mean % survival for all scions across all four *Phaseolus* species. Similar studies in peas with 250 grafts (25 combinations) revealed no significant effect of 5 different root stocks on any of the four different scions (including wild species) for mean % survival of grafts and seed yield, but influence of root stocks on days to flowering was observed. An average 75-100 % survival of grafts was obtained through grafting. Grafting techniques were standardised for faba bean by using 4 scions and 4 root stocks (200 grafts, 20 combinations). Significant differences for the % mean survival of scion grafts were observed for various root stocks. An average of 60% success on grafting was obtained. The standardized grafting techniques using compatible root stocks for grafting in these three major pulse crops appears to be an excellent breeding tool. It can be used for seed multiplication of early generation segregating breeding material, for multiplication of clones for screening and for perpetuation of interspecific hybrids. Rooting techniques with various rooting media and rooting hormones were also studied for these crops and the seed yield

through rooting and grafting were compared. Grafting techniques were found to be superior compared to rooted cuttings in all the three crops for seed yield.

2. Introduction The genetic improvement effort for pea, common bean and faba bean includes introduction of genes from unadapted parents and wild species as a way to improve productivity, reduce input costs and increasingly, improve specific quality traits. We are also beginning to use molecular marker-assisted approaches and expect that for the breeding system used at the CDC (high proportion of multiparent crosses), the most important generation for screening individual plants will be the F_1. The three barriers that we cannot overcome are: (1) the low number of F_1 seeds produced per pollination in large-seed legumes compared to oilseed and cereal crops; (2) the low seed increase ratio of large-seeded legumes– a typical F_1 plant often produces only 50-100 seeds under ideal conditions (3) the difficulty in rooting plants derived from double haploid culture (peas) or interspecific hybrids produced through in vitro techniques (common bean). Perpetuation of rare breeding material becomes very difficult without easy availability of suitable laboratory techniques. The constraints extend beyond the species for common bean where we consider the use of wild species as gene donors for specific traits like frost tolerance and disease resistance. Materials produced either through wide hybridizations or through intervarietal hybrizations have to be screened for multiple breeding objectives. Ideally this would be done as early possible to eliminate undeisrable phenotypes before spending significant time and effort on them.

Alternative techniques are essential for producing a large number of clones of any hybrid in order to have a large population within a specified time schedule. We know that if we could develop a set of highly reliable propagation techniques we would be able to use a lower number of F_1 seeds (reducing costs) needed to produce a larger number of seeds (increased efficiency). This is especially important for those F_1 plants with genetic combinations that can be identified by markers to be superior. Clones produced through asexual reproduction will allow the breeder to screen for multiple traits in the F_1 generation and a large remnant population could be maintained for further evaluation.

Grafting, rooted cuttings and micro-propagation are the most widely used propagation techniques in many tree legumes and horticultural crops. Rooting through stem cuttings is generally not successful in these crops especially in faba bean, or in interspecific hybrids between common bean and *P. acutifolius* and *P. angustissimus*, species which are sources of genes for many useful

traits (for example, frost tolerance and disease resistance). Standardization of an efficient and effective alternative asexual propagation technique will increase the breeding efficiency through mass multiplication of early generation clones in dry bean, faba bean and pea. We are currently working on the transfer of various qualitative characters from wild progenitors to cultivated varieties of pea, bean and faba bean. This involves large scale multiplication and maintenance of rare hybrids with genotypes from the primary and secondary gene pools through asexual propagation for further breeding procedures. We have already successfully developed a set of techniques for lentil and chickpea breeding and are now regularly using them in specific situations. For example, we have successfully used micro grafting to rescue lentil interspecific hybrids for transfer of disease resistance.

Perpetuation and further breeding improvement are currently stalled in our interspecific hybrids in bean, produced for transferring useful traits from the wild gene pool, because of problems in rooting. A new technique for producing double haploid lines in field pea is being tried in the breeding program, but we anticipate a need for grafting, because legume double haploids tend to have problems rooting in tissue culture. Alternative techniques are essential for producing a large number of clones of any hybrid / tissue culture derived double haploids in order to have a large population within a specified time schedule. Standardization of an efficient and effective alternative asexual propagation technique will increase the breeding efficiency through mass multiplication of early generation clones in dry bean, faba bean and pea.

The specific objectives were:

(a) Identification of compatible root stocks for intervarietal, intraspecific and interspecific scions derived through either in vivo or in vitro breeding,

(b) developing standard protocols of techniques for clonal / asexual propagation methods for mass multiplication of breeding materials, and

(c) development of rooting techinques for mass propagation.

3 Material, Methods and Experimental Designs

3.1 Grafting Method.

Grafts were produced using the wedge graft method. Just prior to the first leaf emergence, the root stock was excised below the cotyledons. A vertical slit was made with a sharp blade. Scions with a healthy shoot tip and one internode were cut from the donor plant and a "V"-shaped cut was made at the end. The scion was immediately inserted into the root stock slit and the graft union was wrapped with a stretched piece of Parafilm® to prevent desiccation. The pot carrying the graft was placed on an inverted larger pot covered with a Sun bag® (Sigma, 44.0cm x 20.5 cm; with a 24 mm 0.02 μm filter) and secured using a rubber band to maintain humidity. Bags were opened partially upon the establishment of the grafts and were fully removed once the grafts scions had developed at least one new internode. After the removal of bags, the grafts were carefully transplanted into 1 gallon pots filled with standard potting soil (Sunshine Grow Mix # 4, Sun Gro Horticulture Canada Ltd., AB, Canada.). Parafilm tapes were left at the graft unions until harvest.

The terminal shoots of scion donor plants were clipped off after 3 weeks to encourage the growth of auxiliary shoots for more scion clippings for further grafting. Interspecific F_1s (sterile hybrids) used as scions were trimmed and maintained to produce enough healthy scions for grafting. The root stocks of pea and faba bean were grown directly in 1 gallon pots for grafting and thus no transplanting after grafting was done for faba bean and pea.

As an example of the experimental design used, a total of 300 grafts were produced (10 grafts per treatment combination) for common bean. These were analyzed as a randomized complete block in the growth room.

3.2 Rooting Method.

For rooted cuttings, the cuttings with at least 2 internodes (one node for rooting) were clipped from seedlings and slight injury on the node was made with a razor blade. The cuttings were dipped in rooting hormone powder / hormone liquid (TakeRoot, IBA, Schultz, USA) and planted immediately in to a 4" pot filled with standard Sunshine grow mix # 3 (Sun Gro Horticulture

Canada Ltd., AB, Canada). Pots were randomly arranged in trays and covered with a plastic lid to maintain humidity. Removal of the plastic lid and transfer of established rooted cuttings from a 4" pot to a 1 gallon pot were done as described for grafts above.

3.3 Plant Materials

3.3.1 Grafting Experiments with Common Bean

The following table lists all scion and root stock genotypes used in the experiments.

Table 1. List of scions and root stocks used for grafting experiments for common bean and wild *Phaseolus* species.

SCIONS		SOURCE
1	*P. acutifolius* W6 15578	USDA, Pullman
2	*P. angustissimus* PI 535272	USDA, Pullman
3	F_1 - *P. acutifolius* W6 15578 x *P. angustissimus* PI 535272 (interspecies F_1)	CDC breeding program, University of Saskatchewan
4	*P. vulgaris* (*Pv* intervarietal F_1)	CDC breeding program, University of Saskatchewan
5	*P. vulgaris* 'CDC Whitecap'	CDC breeding programme, University of Saskatchewan
ROOT STOCKS		
1	*P. coccineus* – 'Pole'	Early's Feed and Seed, Saskatoon
2	*P. vulgaris* 'ICA Pijao'	Centro International de Agriculture Tropical (CIAT), Cali, Colombia
3	*P. vulgaris* 'CDC Pintium'	CDC breeding programme, University of Saskatchewan
4	*P. vulgaris* 'NY5-161'	Cornell University
5	*P. vulgaris* 1533-15	CDC breeding programme, University of Saskatchewan
Control: Self grafts of root stocks		

Experimental design: RCBD; Treatments – 30; Replication -2; grafts or rooted cuttings per replication - 10; Site: Controlled growth chambers in the U of S phytotron using standard day length (12 hr) and temperature settings (26 C – day, 18 C – night)

3.3.2 Grafting vs Rooted Cuttings Experiments for Common Bean

NY5-161 genotype; Replication 3; design – RCBD; Location – U of S phytotron.

3.3.3 Grafting Experiments for Pea

Scion genotypes

1) *Pisum sativum* ssp *abyssinicum* 358616 (wild);
2) *P. sativum* ssp *elatius* 560056 (wild);
3) Intervarietal F_1 (Tudor x CDC Striker);
4) *P. sativum* cultivar (CDC Rocket);
5) Self grafts as control

Root stock genotypes

1) Alfetta
2) CDC Centennial
3) CDC Gold
4) Cooper
5) Nitouche

Experimental design: RCBD

Treatments – 30

Replications - 2

Grafts per replication - 10

Site: Controlled growth chambers, Uof S phytotron

3.3.4 Grafting vs Rooted Cuttings Experiment for Pea

Genotype: CDC Rocket

Experimental design: RCBD

Treatments – 2

Replications – 5

Grafts or rooted cuttings per replication - 10

Site: Controlled growth chambers, UofS phytotron

3.3.5 Faba Bean Grafting Experiment

Root stock genotypes: FB 25-26 ZT; FB 143-10 ZT; FB 18-20; FB 21-13 – all are UofS breeding lines.

Scion genotypes: Gloria ZT; Disco ZT; FB 20-5; FB 25-21; self grafts as control

3.3.6 Faba Bean Experiment for Standardising Medium for Rooted Cuttings

Genotype - Disco

Rooting media – 3 - soil; peat moss (jiffy pots); fine silica

Rooting hormone – #3 and liquid gel commercial rooting hormone (0.1% IBA concentration)

Experimental design: RCBD

Treatments – 6

Replications – 4

Grafts or rooted cuttings per replication – 10

Site: Controlled growth chambers, UofS phytotron

3.3.7 Statistical Analysis for All Experiments

SAS Proc GLM procedure, Type III analysis

4. Results

Results are presented for each set of experiments on a crop by crop basis.

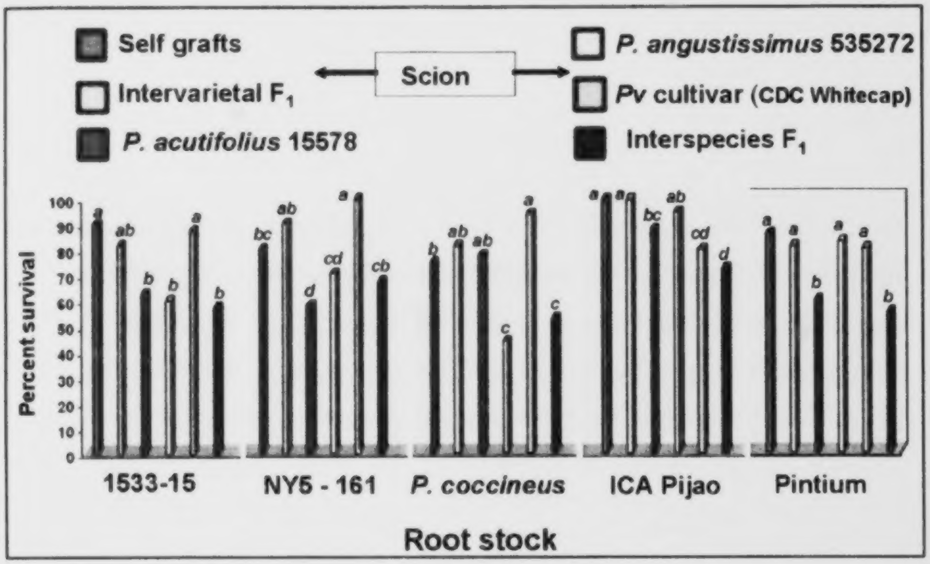
4.1 Common Bean Experiments

4.1.1 Grafting Experiments with Common Bean

A significant influence of root stock (*P. vulgaris* and *P. coccineus)* and an interaction with scions of different species was observed for the percent survival of grafts only, but not for seed yield or pod yield (Fig.1). Use of various scions across three *Phaseolus* species clearly indicated that 'ICA Pijao' was the best root stock for grafting with high mean percent survival for scions from various breeding strategies (Fig 1). When used in interspecific crossing, this Mesoamerican line has been shown to be a non-carrier of the incompatibility gene which appears to participate in sub-lethal interactions in other interspecies hybrids and leads to dwarfism, stunted growth and loss of half of the progeny in the first interspecific cross (Parker and Michaels 1986; Singh and Gutierrez 1984). 'ICA Pijao' has been widely and successfully used as a female parent in interspecies crosses involving common bean. Since 'ICA Pijao' is the carrier of the viable genes for wide cross compatibility, it is hypothesized that high success of grafting with scions of other *Phaseolus* species may also be due to the influence of the compatibility genes. Our grafting studies clearly showed that 'ICA Pijao' is a compatible genotype for overcoming asexual barriers for grafting across the *Phaseolus* genome.

Parental compatibility of different species along with the interspecific hybrids showed the feasibility of using grafting techniques with the best root stock either in intervarietal or interspecific hybridizations. *Phaseolus coccineus* is genetically more closely related to *P. angustissimus* and *P. acutifolius* than it is to *P. vulgaris*, and was therefore expected to be more asexually compatible for grafting. It was found, however, to be comparatively less compatible with *P. angustissimus* scions and the interspecies F_1 scions of *P. angustissimus* and *P. acutifolius*. This may be due to variation in the vascular pattern in *P. coccineus* stems leading to failure of graft unions. Although *P. coccineus* showed similar results to 'ICA Pijao' for survival of grafts for P. *vulgaris* scions, it is not recommended considering the technical difficulties in timely removal of hypogeal shoots of *P. coccineus*, which compete with the growth of the grafted scions.

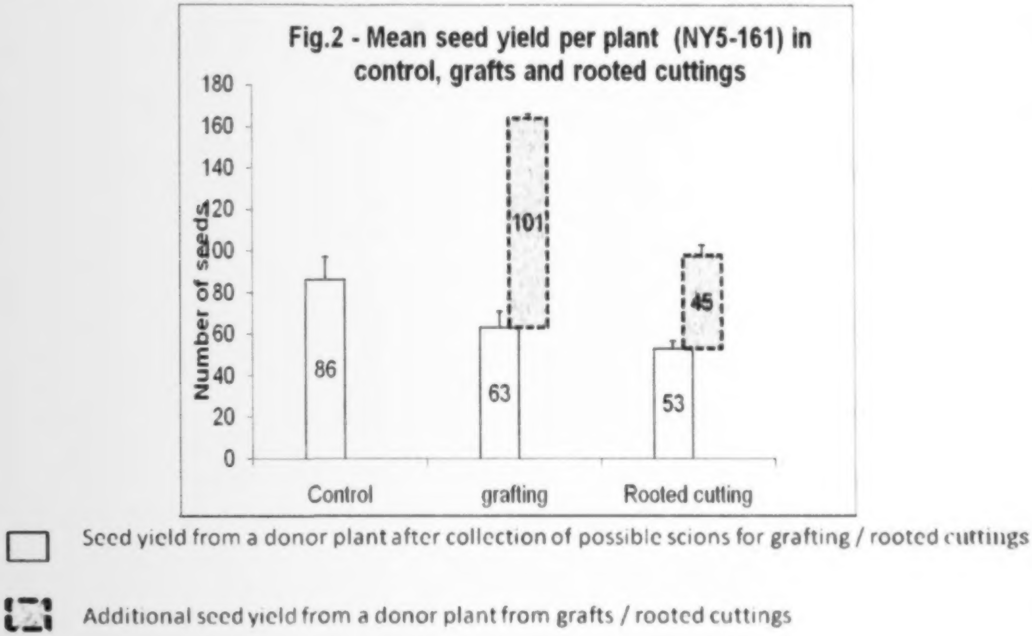
Fig 1. Percent survival of scions of different species on various root stocks for common bean



4.1.2 Rooting vs. Grafting Experiment – Common Bean

The common bean line NY5-161 has a determinate growth habit and it was only possible to propagate three grafts or two rooted cuttings from a single donor plant before it reached the reproductive stage under the light and temperature conditions used in the study. . A significant reduction in days to flower was observed for the grafted cuttings compared to rooted cuttings. NY5-161 control plants yielded an average of 86 seeds per plant. There was a significant increase in the amount of seed obtained per donor plant through grafting compared to rooted cuttings or control plants ($p \leq 0.001$) (Fig 2). Seed multiplication derived from a single NY5-161 grafted scion yielded an average of 78 seeds more than a NY5-161 control plant (63 from the donor plant plus 101 more from the grafted cuttings). Rooted cutting per plant yielded, on average, only 7 more seeds (53 from the donor and 45 more from the rooted cuttings. (Fig 2). This is important for breeding purposes, because increasing the success of survival of cuttings for clonal propagation or higher seed multiplication can be important for common beans with determinate growth habit. Seem multiplication ratios for plants with determinate growth habit are often much lower that for indeterminate types, where the growth of auxiliary shoots could be

triggered to produce many cuttings. As such, determining the best method for obtaining the maximum amount of seed from a few cuttings is critical. In this study, the grafting method was a better tool for perpetuation and multiplication compared to the rooted cutting method. .



In conclusion, grafting shoots of different *Phaseolus* breeding materials can be successfully used as a tool to increase the efficiency of a bean breeding program. 'ICA Pijao' is recommended as a best root stock for grafting from across the *Phaseolus* genus.

4.2 Pea Experiments

4.2.1 Grafting Experiments with Pea

There was no significant influence of root stock on various scions upon grafting for the mean percent survival of grafts and seed yield per graft. Mean percent survival of grafts ranged from 75 - 100 % (Table 2). This indicated that, any of the root stocks (Alfetta, CDC Centennial, CDC Golden, Cooper, Nitouche) can be successfully used as a root stock for grafting scions of pea

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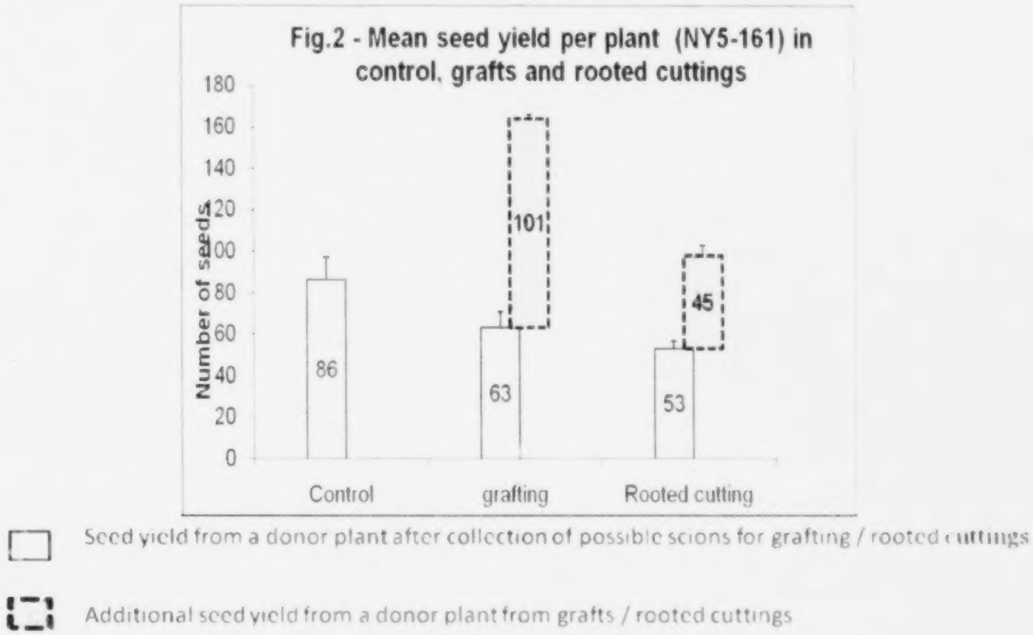

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4.2 Pea Experiments

4.2.1 Grafting Experiments with Pea

There was no significant influence of root stock on various scions upon grafting for the mean percent survival of grafts and seed yield per graft. Mean percent survival of grafts ranged from 75 -100 % (Table 2). This indicated that, any of the root stocks (Alfetta, CDC Centennial, CDC Golden, Cooper, Nitouche) can be successfully used as a root stock for grafting scions of pea

cultivars, interspecific hybridizations or for rescuing *in vitro* generated shoots through double haploid breeding.

Significant influence of root stock ($P \leq 0.001$) and root stock x scion interaction ($P \leq 0.001$) was observed for days to flowering Though there was variation in days to flowering in scion genotypes upon grafting depending on the root stock genotype, no difference in yield was obtained (Table 2).

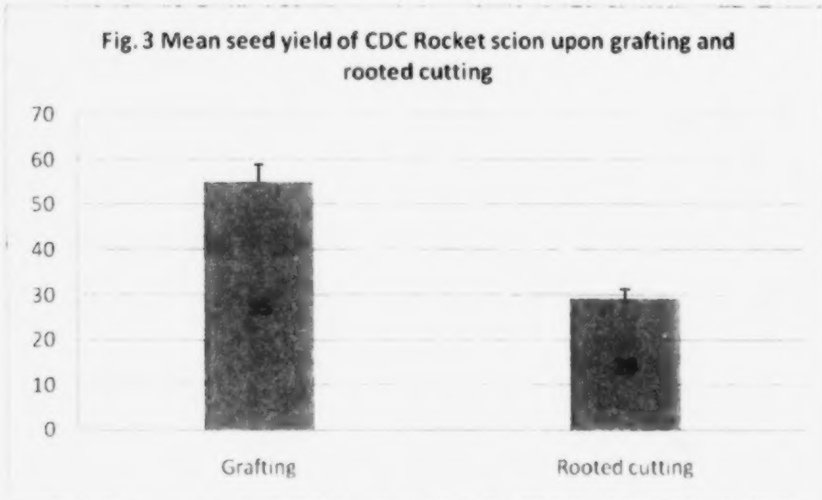
Table 2: Mean days to flowering in various scions upon grafting with various root stocks of pea.

	Root stock				
Scions	CDC Centennial	Nitouche	CDC Golden	Cooper	Alfetta
CDC Rocket	28^{ab}	26^{b}	29^{ab}	26^{b}	31^{a}
Intervarietal F_1 (Tudor x CDC Striker)	19^{b}	32^{a}	31^{a}	30^{a}	16^{b}
Wild *P. sativum* ssp *abyssinicum* 358616	26^{b}	17^{c}	31^{a}	32^{a}	25^{b}
Wild *P. sativum* ssp *elatius* 560056	26^{b}	26^{b}	33^{a}	27^{b}	26^{b}

4.2.2 Grafting vs Rooted Cutting Experiment – Pea

Seed yield of scion genotype CDC Rocket varied significantly ($P \leq 0.01$; Fig 3) for grafting compared to rooted cuttings. Comparison of grafts vs. rooted cuttings for seed yield indicated that a single grafted scion produced, on average, 55 seeds whereas rooted cuttings yielded 29 seeds (Fig 3). The donor plant scion yielded an average seed yield of 74 seeds after the collection of a single scion. A single control plant CDC rocket yielded 80 seeds. Thus, an additional seed yield of 44 seeds per plant was obtained through grafting compared to 23 seeds through rooted cuttings.

The results show clearly that grafting of pea can be used to improve seed multiplication ratio in pea breeding. This may be particularly useful for future research involving crosses with other pea species, especially for genetic and genomic studies where it is useful to enlarge the size of F2 populations derived from a single pollination event.



4.3 Faba Bean Experiments

4.3.1. Grafting Experiments with Faba Bean

Significant difference for the percent means survival of scion grafts were observed for various root stocks (Fig 4). Root stock FB 21-13 was found to be the best root stock with at least 60% of mean survival of grafts for all the scions. Root stock FB143-10 ZT was found to be least compatible for grafting including self grafts. All the root stocks except FB 21-13 were found to be less compatible for self grafting compared to grafting with other donor scions. This provides some evidence of genotypic influence, more like the results of the common bean experiments.

Fig. 3 Mean seed yield of CDC Rocket scion upon grafting and rooted cutting

4.3 Faba Bean Experiments

4.3.1. Grafting Experiments with Faba Bean

Significant difference for the percent means survival of scion grafts were observed for various root stocks (Fig 4). Root stock FB 21-13 was found to be the best root stock with at least 60% of mean survival of grafts for all the scions. Root stock FB143-10 Z1 was found to be least compatible for grafting including self grafts. All the root stocks except FB 21-13 were found to be less compatible for self grafting compared to grafting with other donor scions. This provides some evidence of genotypic influence, more like the results of the common bean experiments.

Fig 4: Percent mean survival of faba bean grafts up to maturity in various scions and root stocks

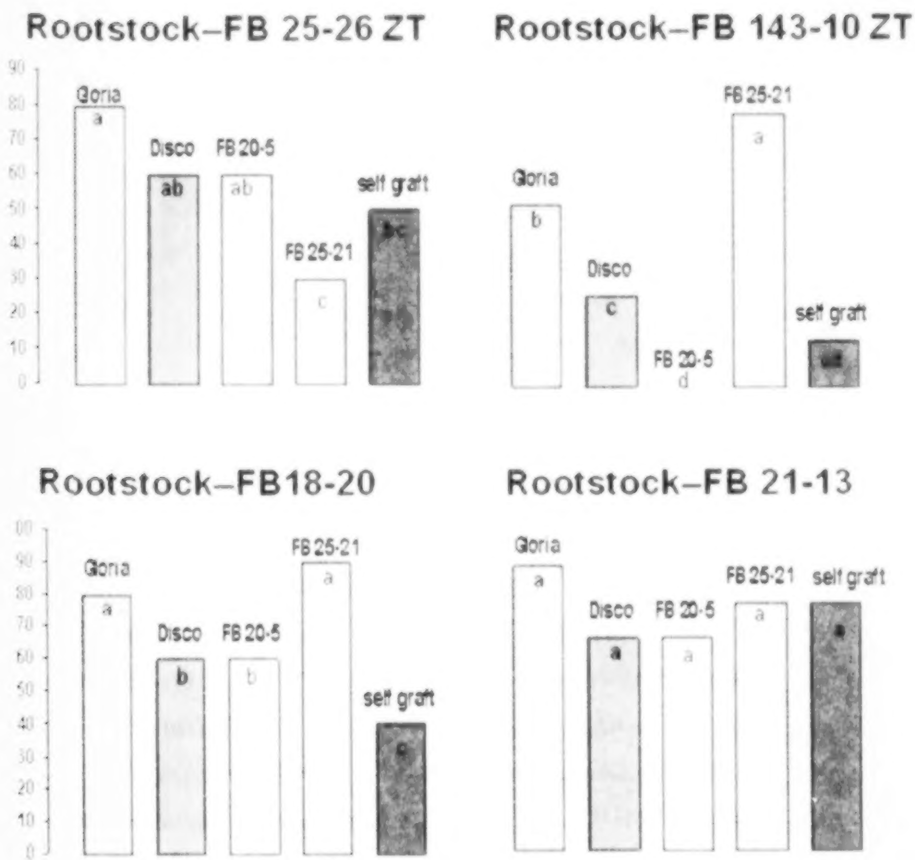
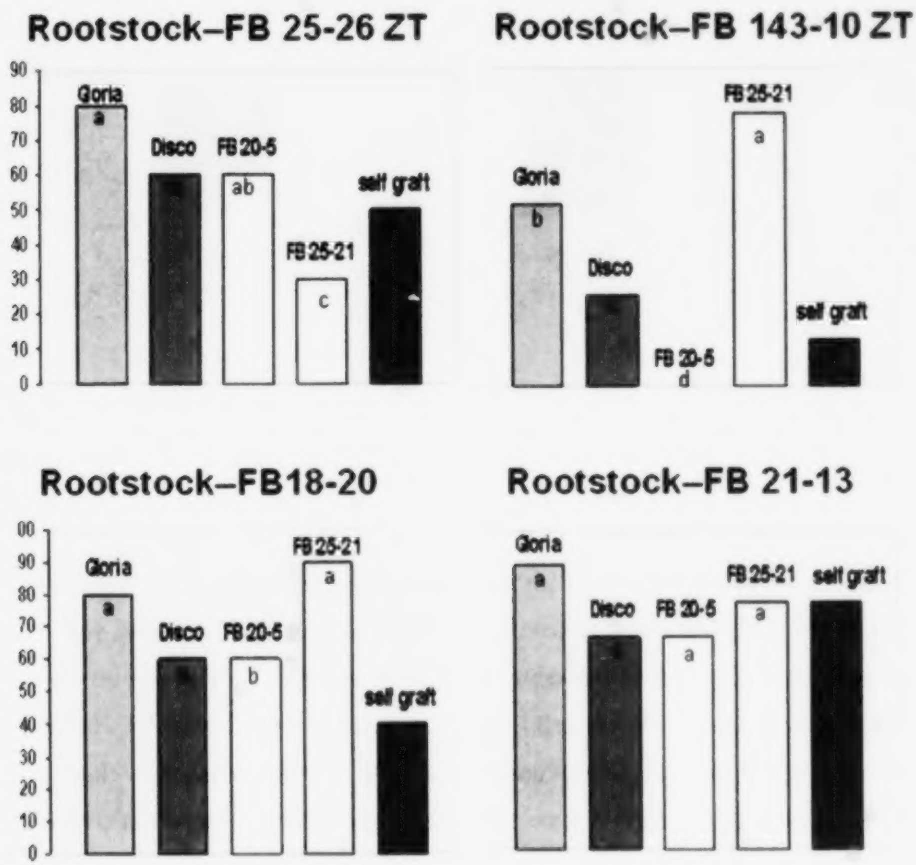


Fig 4: Percent mean survival of faba bean grafts up to maturity in various scions and root stocks



Faba bean plants were harvested at physiological maturity and data on seed yield per graft were analysed using SAS Proc Genmode Type III analysis (Table 3). Significant effects of rootstocks on graft scions ($p \leq 0.01$) and root x scion interaction ($p \leq 0.05$) for mean seed yield were observed. Rootstock FB 18-20 was found to be most suitable rootstock for all the scions except for genotype 'Gloria'. It had low yield when self-grafted. FB 25-66 was found to be the best compatible rootstock for the scion genotype Gloria.

Table 3: Average seed yield per scion ("Disco") on grafting with various rootstocks

Rootstock	Gloria ZT	Disco ZT	FB 20-5	FB 25-21	control
FB 25-66 ZT	34 ^{a}	12 ^{b}	11 ^{b}	12 ^{b}	24 ^{ab}
143-10 ZT	11 ^{ab}	11 ^{ab}	0 ^{c}	16 ^{a}	14 ^{a}
FB 18-20	26 ^{a}	16 ^{ab}	14 ^{ab}	20 ^{ab}	8 ^{b}
FB 21-13	14 ^{a}	9 ^{a}	14 ^{a}	14 ^{a}	11 ^{a}

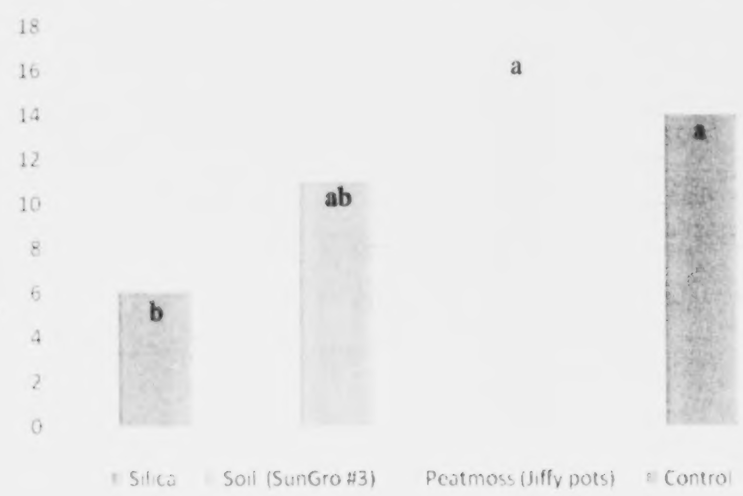
Asexual cross- and self-incompatibility issues were noticed among the four rootstock and four scion genotypes used in this study. Screening of a larger number of rootstock genotypes for grafting may be helpful to discover the most highly compatible genotype which could be recommended for regular use breeding programs.

4.3.2 Standardising Rooting Techniques for Faba Bean

Due to the compatibility issues observed in the grafting protocol assessment, various rooting media for production of rooted cuttings were studied to identify the best medium for successful rooting with high seed yield. Three different rooting media (1: Silica; 2: Soil – SunGro Soil mix #3; 3: Jiffy pots – Peat) along with rooting hormone powder (#3 IBA commercial rooting hormone, based on our preliminary studies) were used for making rooted cuttings. Results obtained on mean seed yield of rooted cuttings established on different medium are shown below (Fig 5). Even though 100% survival of rooted cuttings was observed in all the methods, Jiffy pot

was found to be the best medium for rooted cutting with high mean seed yield of 17 seeds per plant (Fig 5).

Fig 5. Number of seeds per rooted cutting of genotype 'Disco' on various media for rooting

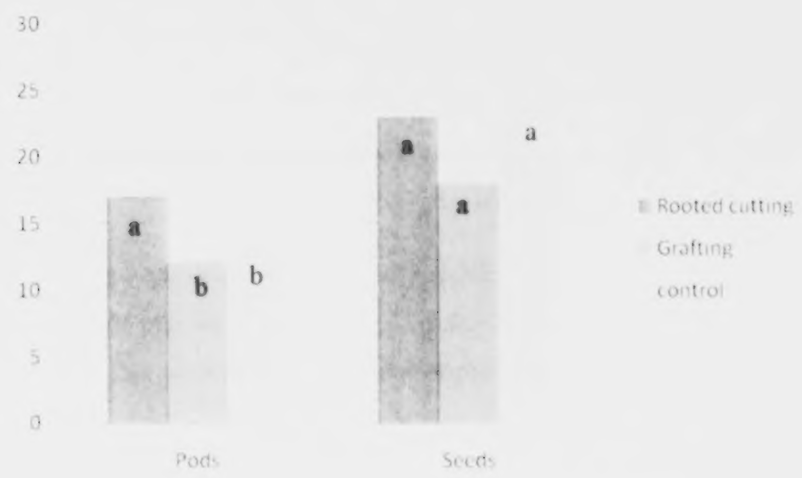


Note: Commercial liquid gel rooting hormone was initially tried as a treatment for rooting. The survival of cuttings was greatly reduced due to the decay of tissues above the soil level (data not included). Thus rooting hormone #3 along with the various media was used to standardise the suitable medium for rooted cuttings.

4.3.3 Grafting vs Rooted Cutting Experiment – Faba Bean

Similar to the studies in common bean and pea, an experiment was conducted to identify the feasibility of using rooted cuttings (on jiffy pots) or grafts for mass seed multiplication in faba bean. Average mean seed yield and pod yield obtained through rooted cuttings and grafts are shown in Fig 6. Thus it is recommended that higher seed yield could be obtained through properly rooted cuttings.

Fig 6. Mean seed and pod yield per plant of the faba bean (genotype 'Disco') for rooted cuttings and grafts to rootstock 'FB 18-20'.



Though number of pods per plant on rooted cutting was higher than the grafts, seed filling at the end of the physiological maturity was poor and thus a lesser seed yield was obtained.

Compatibility issues observed in faba bean grafting could be sorted out by screening a large number of rootstocks. Though grafting is a successful technique for perpetuation of hybrids; rooted cuttings is recommended for clonal or seed multiplication. Rooting in jiffy pots was found to be the best method with high seed yield per plant.

5. Conclusions and Recommendations:

These experiments conclusively showed that grafting techniques can be successfully used as useful tools for use in breeding and genetic study applications to increase efficiency of seed multiplication of valuable breeding materials for common bean, pea and Faba bean.

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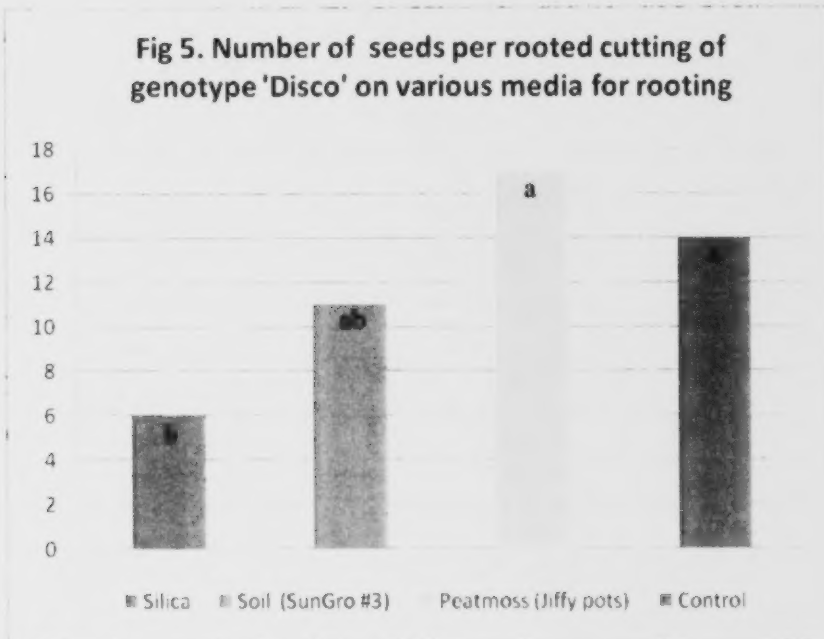

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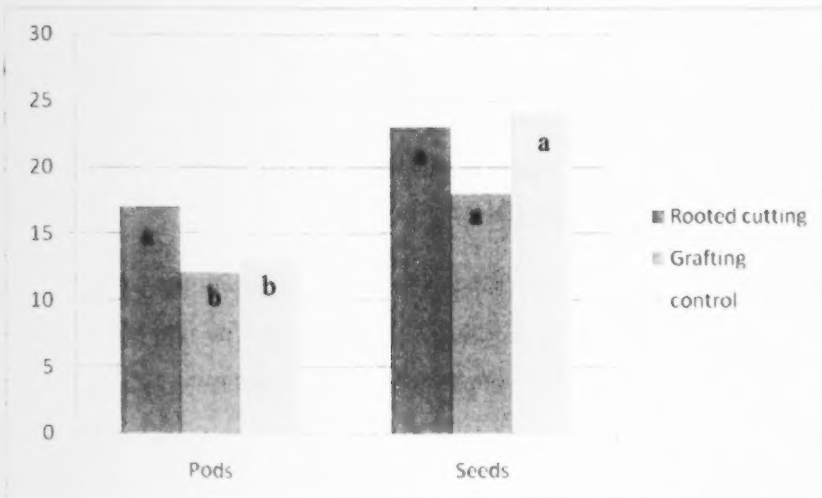

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Compatibility issues observed in faba bean grafting could be sorted out by screening a large number of rootstocks. Though grafting is a successful technique for perpetuation of hybrids; rooted cuttings is recommended for clonal or seed multiplication. Rooting in jiffy pots was found to be the best method with high seed yield per plant.

5. Conclusions and Recommendations:

These experiments conclusively showed that grafting techniques can be successfully used as useful tools for use in breeding and genetic study applications to increase efficiency of seed multiplication of valuable breeding materials for common bean, pea and Faba bean.

- 'ICA Pijao' is recommended as the best root stock for grafting from across the *Phaseolus* genus and for interspecific hybrid scions. Significant root stock x scion interactions were observed for grafting.

- Grafting was shown to be an efficient tool for enhancing the rate of seed multiplication for common bean plants with determinate growth habit. Increasing the success of survival of cuttings for clonal propagation or higher seed multiplication for determinate type beans is more critical compare to indeterminate type bean, where the growth of auxiliary shoots could be triggered to produce many cuttings. The grafting method produced more seeds compared to the rooted cuttings.

- The grafting technique also increased the seed multiplication potential compared to rooted cuttings for field pea. Grafting technique was proved to be a better technique in pea and there was no specific compatibility issue in pea root stock genotypes, even across sub-species.

- Faba bean seed multiplication ratios were increased for rooted cuttings compared to grafting. Though the grafting technique was successful, selection of compatible rootstock for vigorous scion growth and further seed multiplication was found to be critical

- Faba bean experiments for grafting showed interaction between root stocks and scions, like common bean. .

In conclusion, grafting methods can be recommended as a tool in breeding programs for all three crops. Useful applications are envisaged in future for:

- Increasing seed multiplication ratios of rare and difficult F_1 hybrids within and between species.

- Developing large F2 populations for genetic and genomic research applications.

- Enhancing survivability of interspecific hybrid scions.

6. Future Research and Development Indications

Based on our studies with faba bean genotypes, further studies should be taken up with more root stock genotypes. There is evidence of asexual self incompatibility. It would be possible to study the relationship of ZT or regular type root stocks and their interaction upon grafting with various scions. It would be useful to identify a "universal" root stock in the same way that 'ICA Pijao' was found to be universally useful as a root stock for common bean.

As a small experiment with faba bean we tried to graft lentil onto faba bean root stocks. Our preliminary trials on grafting lentil scions onto faba bean root stocks were very promising. Some lentil grafts completed their life cycle and successfully produced normal seeds which subsequently had normal germination. Faba bean was selected as root stock for lentil scions for wedge grafting for the ease of handling. The thick and sturdy stems of faba bean root stocks are much easier to use compared to the thin and flexible lentil stems. It is also suggested that studies be undertaken to identify a standard faba bean root stock for lentil species. This would be particularly useful for interspecific breeding projects for lentil. This research will be incorporated into the proposed Industrial Research Chair for Genetic Improvement of Lentil.

A scientific publication based on this research will be drafted for submission to a peer reviewed scientific journal to allow the results to become available to the pulse crop research community.

7 Acknowledgements

The Saskatchewan Ministry of Agriculture and the Agriculture Development Fund were acknowledged in all presentations where this research was discussed. (Presentation details are given in Section 10 below.

8 Literature Cited

Parker JP, Michaels TE (1986). Simple genetic control of hybrid plant development in interspecific crosses between *Phaseolus vulgaris* L., and *P. acutifolius* A. Gray. Plant Breeding 97: 315-323.

Singh PS, Gutierrez JA (1984) Geographical distribution of DL1 and DL2 genes causing hybrid dwarfism in *Phaseolus vulgaris* L., their association with seed size and their significance to breeding. Euphytica 33:337-345.

9 Appendices

All data were summarized in the form o Tables and Figures presented in the appropriate sections of this report. Poster presentations are attached as PDF files.

10. Communications Report and Other Requirements

10.1 Extension Meetings

Research and development activities were presented at Pulse days 2008, Saskatoon and Pulse Days 2009. Saskatoon and presented posters on the developments of this research.

10.2 Conference Presentations

1. ***6th European Legume Conference, Nov. 2007***, Lisbon Portugal – Presented a poster on " Grafting as tool in large seeded legume breeding" (Valarmathi Gurusamy, Kirstin Bett Tom Warkentin and Albert Vandenberg)
2. ***BIC 2007***– Presented and published 2 page paper and a poster on "Grafting as tool in bean interspecies breeding" (Valarmathi Gurusamy, Kirstin Bett and Albert Vandenberg)
3. ***Pulse Days 2008*** - Presented poster on "Grafting as tool in large seeded legume breeding" (Valarmathi Gurusamy, Kirstin Bett, Tom Warkentin and Albert Vandenberg)
4. ***Pulse Days 2009*** - Presented poster on "Asexual propagation techniques to accelerate breeding efficiency in major legume crops" (Valarmathi Gurusamy, Kirstin Bett, Tom Warkentin and Albert Vandenberg)
5. Submitted for presentation at the ***International Conference on Grain Legumes, at Kanpur, India, Feb 14-16 2009.*** " Grafting as a breeding tool for genetic enhancement in legumes with a special emphasis on interspecific breeding" (Valarmathi Gurusamy, Kirstin Bett, Tom Warkentin and Albert Vandenberg)

PDF copies of poster presentations are attached to this report.

10.3 Invited Presentations

This project was featured as an invited presentation to the College of Agriculture faculty and staff at Tamil Nadu Agricultural University in Coimbatore, TN in February 2009.

10.4 Scientific Publications

A manuscript with the working title "Grafting as a breeding tool in common bean breeding" for the submission to the journal" Euphytica" is in preparation.

A second manuscript publishing the work on field pea and faba bean is also planned.

10.5 Personnel

Salary for a Professional Research Associate wasg paid from this project fund. A University of Saskatchewan financial report is provided as an attachment to the report.

10.6 Equipment

No equipment has been purchased.

10.7 Project Developed Materials

Grafting techniques were standardized for common bean, pea and faba bean and ideal root stock genotypes were identified for accelerating breeding strategies.

10.8 Project Photos

Several photos of grafting, techniques and the experiment in growth chamber were taken for use in posters, oral presentations and publications. These can be provided upon request.

11. Expense Statement

Provided by UofS. See attachment (PDF file).

GRAFTING AS A TOOL IN LARGE SEEDED LEGUME BREEDING

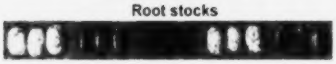
VALARMATHI GURUSAMY, KIRSTIN BETT, TOM WARKENTIN AND BERT VANDENBERG
Dept. of Plant Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8 Canada

Background and Objectives:

Genetic improvement efforts in pea and common bean include the introduction of genes from unadapted parents and wild species. Three common barriers in the breeding strategies of these large seeded legumes are: (1) the number of F_1 seeds produced per pollination, (2) low seed increase ratio and, (3) difficulty in rooting plants derived from interspecific hybrids produced through *in vitro* techniques (bean) or doubled haploid culture (pea). Seed quantity is generally limiting in early generation screening for multiple breeding objectives. Rooting through stem cuttings is generally not successful in these crops especially in interspecific hybrids between common bean and *P. acutifolius* and *P. angustissimus*, which are sources of genes for many useful traits (e.g. frost tolerance and disease resistance). Standardization of an efficient alternative asexual propagation technique will increase breeding efficiency through mass multiplication of early generation clones in dry bean and pea. Thus, our objectives are to standardize grafting techniques and identify suitable root stock for bean and pea so that these techniques can eventually become standard tools in breeding programs.

Materials - Bean

Root stocks



1533-1 NY5-161 ICA Pijao Pintium *P. coccineus*

Scions

1) *Phaseolus acutifolius* WP15578
2) *P. angustissimus* 535272
3) Intervarietal F_1 of *P. vulgaris*
4) *P. vulgaris* cultivar (CDC Whitecap)
5) Interspecies F_1 (*P. acutifolius* x *P. angustissimus*)
6) Self grafts

Methods- Bean

6-7 day old root stocks were cut below the cotyledons and 1-2 node scions were grafted onto them (Fig 2a). High humidity was maintained by covering plant + pot tightly with a polythene bag (Sigma Sun bags; Fig 3b). Bags were slowly opened and removed after observing growth of the scions by one additional node (Fig 3c). Observations on survival of grafts, days to flowering, total number of pods and seeds per graft were recorded on established grafts (Fig 2b, 2c & 3d, 3e). The experiment was conducted in controlled growth chambers with 2 replications.

Results and Discussion (Bean)

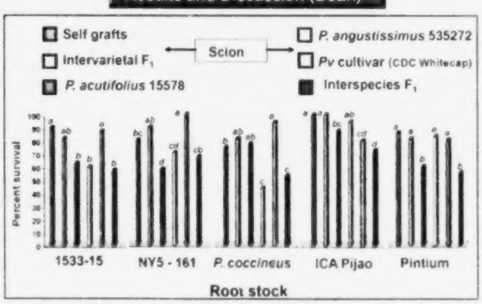


Fig 1. Percent survival of scions of different species on various root stocks in bean

A significant influence of root stock (*P. vulgaris* and *P. coccineus*) and an interaction with scions of different species was observed for the percent survival of grafts only, **but not for seed yield or pod yield.** *P. vulgaris* ICA Pijao was the best root stock for survival of the scions of wild species, interspecific hybrids and intervarietal hybrids upon grafting (Fig 1). Although *P. coccineus* was asexually compatible for grafting with *P. vulgaris* and *P. acutifolius* scions, it was comparatively less compatible with the scions of *P. angustissimus*. Thus, *P. vulgaris* ICA Pijao is recommended as a root stock in bean for improved early generation seed multiplication.

Fig 2: Grafting interspecies F1 hybrid as scion onto *P. vulgaris* ICA Pijao

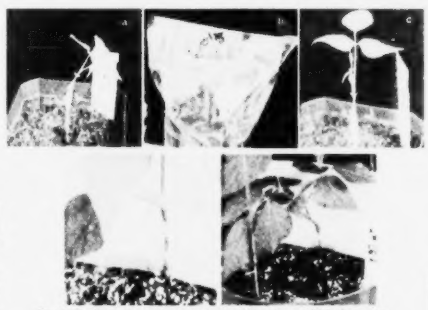


Fig 3. Grafting steps illustrated using *P. vulgaris* CDC Whitecap as scion and *P. vulgaris* NY5-161 as root stock

Fig 4: a & b - Wild pea scions grafted on Nitouche; c & d - CDC Rocket scion grafted on Nitouche

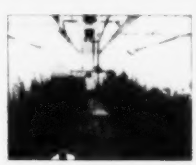
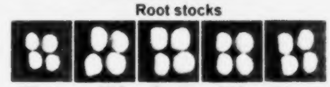


Fig 4e: Pea grafts growing under controlled conditions

Future directions: **The best root stocks identified in bean and pea will be compared with the rooted cuttings for seed yield. The ideal root stock and the ideal stage for grafting will be recommended. Similar experiments will also be conducted in faba bean.**

Materials - Pea

Root stocks



Alfetta CDC Centennial Cooper CDC Golden Nitouche

Scions

1) *Pisum sativum* ssp *abyssinicum* 358616 (wild)
2) *P. sativum* ssp *elatius* 560056 (wild)
3) Intervarietal F_1 (Tudor x CDC Striker)
4) *P. sativum* cultivar (CDC Rocket)
5) Self grafts

Methods- Pea

A total of 25 combinations of grafts (5 root stocks and 5 scions) were produced as described in bean, except that grafting onto the root stock occurred above the 2nd-3rd node (Fig 4)

Preliminary Results and Discussion (Pea)

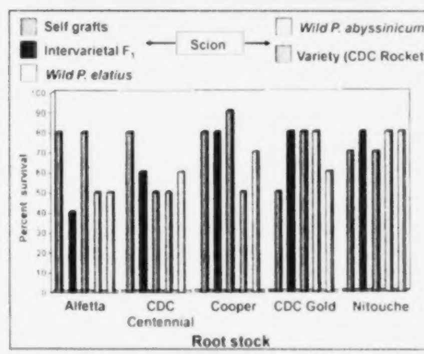


Fig 5. Percent survival of scions of different species on various root stocks in peas

Preliminary results on success of grafting on peas showed a similar trend as observed in beans. Data on % survival of grafts based on one replication is presented here to indicate the trend. Root stock Nitouche gave the maximum pooled percent survival of grafts (76%) with diverse scions (Fig. 5) followed by Cooper (74%). This experiment will be repeated and further observations on seed yield and percent survival will be analysed upon completion.

Acknowledgements

SASKATCHEWAN pulse Growers
Saskatchewan Agriculture and Food

GRAFTING AS A TOOL IN PULSE CROP BREEDING

Valarmathi Gurusamy, Kirstin Bett, Tom Warkentin and Bert Vandenberg
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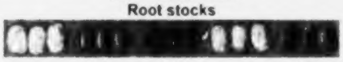
Background and objectives:

Genetic improvement efforts in pulse crops like common bean, faba bean, pea and lentil include the introduction of genes from unadapted parents and wild species. Major barriers in breeding strategies for these crops are: (1) the low number of F_1 seeds produced per pollination, (2) the low seed increase ratio, and (3) the difficulty in rooting plants derived from doubled haploid culture (pea) or interspecific hybrids produced through *in vitro* techniques (bean and lentil). Seed quantity is generally limiting in early generation screening for multiple breeding objectives. Rooting through stem cuttings is generally not successful in these crops, especially in interspecific hybrids between common bean and its wild species and lentils wide hybrids, which are sources of genes for many useful traits (e.g., frost tolerance and disease resistance). Standardization of an efficient alternative asexual propagation technique will increase breeding efficiency through mass multiplication of early generation clones in these crops. Thus, our objectives are to standardize grafting techniques and identify suitable root stock genotypes for common bean, pea, faba bean and lentil so that these techniques can eventually become standard tools in breeding programs.

Bean Grafting

Root stocks



1533-1 NY5-161 ICA Pijao Pintium *P. coccineus*

Scions

1) *Phaseolus acutifolius* WP15578,
2) *P. angustissimus* 535272,
3) Intervarietal F_1 of *P. vulgans*,
4) *P vulgans* cultivar (CDC Whitecap),
5) Interspecies F_1 (*P. acutifolius* x *P. angustissimus*),
6) Self grafts

Total treatments: 30 **Replications:** 2

Results and Discussion: *P. vulgans* ICA Pijao was the found to be the best root stock yielding significantly higher mean percent survival of the scions ($P \le 0.01$) of wild species, interspecies hybrids and intervarietal hybrids upon grafting Thus, *P. vulgans* ICA Pijao is recommended as a root stock in bean for improved early generation seed multiplication

Bean Grafting vs Rooted Cuttings

Materials: Scion - NY5-161 (Determinate type genotype), Rootstock – ICA Pijao

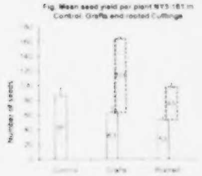
Treatments: Two (grafting and rooted cuttings)

Replication: 3

Method for rooted cuttings scions with 2 nodes were cut, dipped in rooting hormone and planted in soil

Results and Discussion

Fig. Mean seed yield per plant NY5-161 in Control, Grafts and rooted Cuttings



Grafting in NY5-161

Seed yield from a donor plant after collection of possible scions for grafting / rooted cuttings

Additional seed yield from a donor plant from grafts / rooted cuttings

Increasing the success of survival of cuttings for clonal propagation or higher seed multiplication for determinate type beans is more critical compare to indeterminate type bean, where the growth of auxiliary shoots could be triggered for many cuttings. Grafting was a better tool compared to rooted cuttings for determinate type beans perpetuation and multiplication

Methodology for All Crops

Method: Wedge grafting

Number of grafts – 10 per treatment / replication

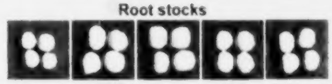
Lentil grafting

Treatments – 6
Replications - 4

Grafting of lentil on their rootstocks is very difficult as the stem girth is very thin which makes the grafting procedure difficult. Faba bean root stocks, which have large thick stems, were tried successfully with lentil scions on a preliminary basis. An experiment is in progress to identify the best faba rootstock for lentil grafting. Initial observations on the establishment of grafts (mean % survival of grafts) indicate that Taboar and Snowbird can be used as rootstocks with 65-70% success for Redberry scions and Taboar and Disco can be used for grafting Plato scions with 45-50% success

Pea Grafting

Root stocks



Alfetta CDC Centennial Cooper CDC Golden Nitouche

Scions

1) *Pisum sativum* ssp *abyssinicum* 358616 (wild pea),
2) *P. sativum* ssp. *elatius* 560056 (wild pea),
3) Intervarietal F_1 (Tudor x CDC Striker),
4) *P. sativum* cultivar (CDC Rocket),
5) Self grafts

Results and Discussion

There were no significant effect of rootstock on any of the scions for mean % survival of grafts, seed yield and days to flowering. An average 75-80% survival was obtained through grafting. Any of the rootstocks above can be successfully used for grafting in cultivated and wild peas and in their interspecific hybrids for seed multiplication and perpetuation

Faba Bean Grafting

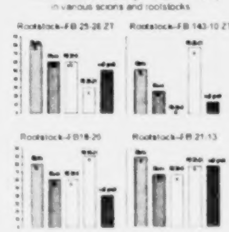
Rootstocks: FB 25-26 ZT, FB 143-10 ZT, FB 18-20 ; FB 21-13

Scions: Gloria ZT, Disco ZT, FB 20-5 , FB 25-21

Total treatments: 16

Results and Discussion

Percent mean survival of grafts up to maturity in various scions and rootstocks



Significant differences for the % mean survival of scion grafts were observed for various root stocks (Fig). Rootstock FB 21-13 was found to be the best root stock with at least 60% mean survival of grafts for all the scions. Rootstock FB143-10 ZT was found to be least compatible for grafting including self grafts. All the rootstocks except FB 21-13 were found to be significantly less compatible for self grafting compared to grafting with other donor scions

Acknowledgements

Saskatchewan pulse Growers

Saskatchewan Ministry of Agriculture

GRAFTING AS A TOOL IN LARGE SEEDED LEGUME BREEDING

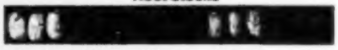
VALARMATHI GURUSAMY, KIRSTIN BETT, TOM WARKENTIN AND BERT VANDENBERG
Dept. of Plant Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8 Canada

Background and Objectives:

Genetic improvement efforts in pea and common bean include the introduction of genes from unadapted parents and wild species. Three common barriers in the breeding strategies of these large seeded legumes are: (1) the number of F_1 seeds produced per pollination, (2) low seed increase ratio and, (3) difficulty in rooting plants derived from interspecific hybrids produced through *in vitro* techniques (bean) or doubled haploid culture (pea). Seed quantity is generally limiting in early generation screening for multiple breeding objectives. Rooting through stem cuttings is generally not successful in these crops especially in interspecific hybrids between common bean and *P. acutifolius* and *P. angustissimus*, which are sources of genes for many useful traits (e.g. frost tolerance and disease resistance). Standardization of an efficient alternative asexual propagation technique will increase breeding efficiency through mass multiplication of early generation clones in dry bean and pea. Thus, our objectives are to standardize grafting techniques and identify suitable root stock for bean and pea so that these techniques can eventually become standard tools in breeding programs.

Materials - Bean

Root stocks



1533-1 NY5-161 ICA Pijao Pintium *P. coccineus*

Scions

1) *Phaseolus acutifolius* WP15578
2) *P. angustissimus* 535272
3) Intervarietal F_1 of *P. vulgaris*
4) *P.vulgaris* cultivar (CDC Whitecap)
5) Interspecies F_1 (*P. acutifolius* x *P. angustissimus*)
6) Self grafts

Methods- Bean

- Total treatments - 30 (5 root stocks and 6 scions)
- No. of grafts made / replication/ treatment – 10
- Grafting method - wedge or cleft type

6-7 day old root stocks were cut below the cotyledons and 1-2 node scions were grafted onto them (Fig 2a). High humidity was maintained by covering plant + pot tightly with a polythene bag (Sigma Sun bags; Fig 3b). Bags were slowly opened and removed after observing growth of the scions by one additional node (Fig 3c). Observations on survival of grafts, days to flowering, total number of pods and seeds per graft were recorded on established grafts (Fig 2b, 2c & 3d, 3e). The experiment was conducted in controlled growth chambers with 2 replications.

Results and Discussion (Bean)

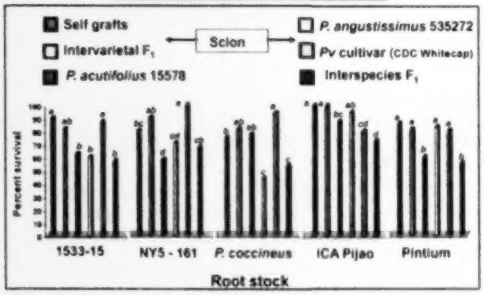


Fig 1. Percent survival of scions of different species on various root stocks in bean

A significant influence of root stock (*P. vulgaris* and *P. coccineus*) and an interaction with scions of different species was observed for the percent survival of grafts only, **but not for seed yield or pod yield.** *P. vulgaris* ICA Pijao was the best root stock for survival of the scions of wild species, interspecific hybrids and intervarietal hybrids upon grafting (Fig 1). Although *P. coccineus* was asexually compatible for grafting with *P. vulgaris* and *P. acutifolius* scions, it was comparatively less compatible with the scions of *P. angustissimus*. Thus, *P. vulgaris* ICA Pijao is recommended as a root stock in bean for improved early generation seed multiplication.

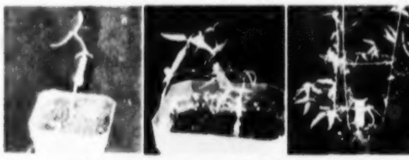


Fig 2: Grafting interspecies F1 hybrid as scion onto *P. vulgaris* ICA Pijao

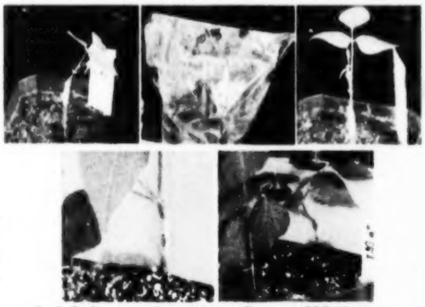


Fig 3: Grafting steps illustrated using *P. vulgaris* CDC Whitecap as scion and *P. vulgaris* NY5-161 as root stock

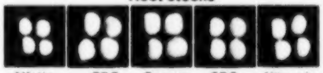
Fig 4: a & b - Wild pea scions grafted on Nitouche; c & d - CDC Rocket scion grafted on Nitouche

Fig 4e: Pea grafts growing under controlled conditions

Future directions: The best root stocks identified in bean and pea will be compared with the rooted cuttings for seed yield. The ideal root stock and the ideal stage for grafting will be recommended. Similar experiments will also be conducted in faba bean.

Materials - Pea

Root stocks



Alfetta CDC Centennial Cooper CDC Golden Nitouche

Scions

1) *Pisum sativum* ssp *abyssinicum* 358616 (wild)
2) *P. sativum* ssp *elatius* 560056 (wild)
3) Intervarietal F_1 (Tudor x CDC Striker)
4) *P. sativum* cultivar (CDC Rocket)
5) Self grafts

Methods- Pea

A total of 25 combinations of grafts (5 root stocks and 5 scions) were produced as described in bean, except that grafting onto the root stock occurred above the 2nd-3rd node (Fig 4).

Preliminary Results and Discussion (Pea)

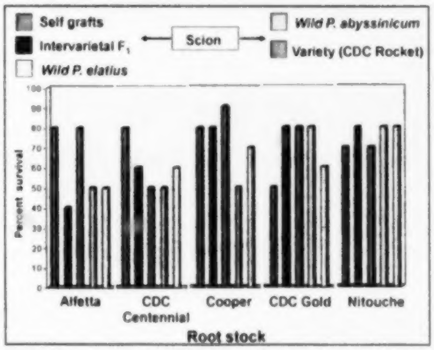


Fig 5. Percent survival of scions of different species on various root stocks in peas

Preliminary results on success of grafting on peas showed a similar trend as observed in beans. Data on % survival of grafts based on one replication is presented here to indicate the trend. Root stock Nitouche gave the maximum pooled percent survival of grafts (76%) with diverse scions (Fig 5) followed by Cooper (74%). This experiment will be repeated and further observations on seed yield and percent survival will be analysed upon completion.

Acknowledgements

SASKATCHEWAN pulse Growers

Saskatchewan Agriculture and Food

GRAFTING AS A TOOL IN PULSE CROP BREEDING

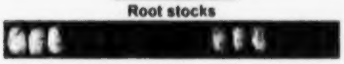
Valarmathi Gurusamy, Kirstin Bett, Tom Warkentin and Bert Vandenberg
Dept. of Plant Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8 Canada

Background and objectives:

Genetic improvement efforts in pulse crops like common bean, faba bean, pea and lentil include the introduction of genes from unadapted parents and wild species. Major barriers in breeding strategies for these crops are: (1) the low number of F_1 seeds produced per pollination, (2) the low seed increase ratio, and (3) the difficulty in rooting plants derived from doubled haploid culture (pea) or interspecific hybrids produced through *in vitro* techniques (bean and lentil). Seed quantity is generally limiting in early generation screening for multiple breeding objectives. Rooting through stem cuttings is generally not successful in these crops, especially in interspecific hybrids between common bean and its wild species and lentils wide hybrids, which are sources of genes for many useful traits (e.g., frost tolerance and disease resistance). Standardization of an efficient alternative asexual propagation technique will increase breeding efficiency through mass multiplication of early generation clones in these crops. Thus, our objectives are to standardize grafting techniques and identify suitable root stock genotypes for common bean, pea, faba bean and lentil so that these techniques can eventually become standard tools in breeding programs.

Bean Grafting

Root stocks



1533-1 NY5-161 ICA Pijao Pintium *P. coccineus*

Scions

1) *Phaseolus acutifolius* WP15578;
2) *P. angustissimus* 535272;
3) Intervarietal F_1 of *P. vulgaris*;
4) *P. vulgaris* cultivar (CDC Whitecap);
5) Interspecies F_1 (*P. acutifolius* x *P. angustissimus*);
6) Self grafts.

Total treatments: 30 **Replications:** 2

Results and Discussion: *P. vulgaris* ICA Pijao was the found to be the best root stock yielding significantly higher mean percent survival of the scions (P≤0.01) of wild species, interspecies hybrids and intervarietal hybrids upon grafting Thus, *P. vulgaris* ICA Pijao is recommended as a root stock in bean for improved early generation seed multiplication.

Bean Grafting vs Rooted Cuttings

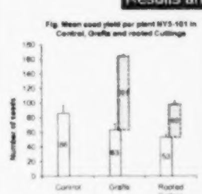
Materials: Scion - NY5-161 (Determinate type genotype); Rootstock – ICA Pijao

Treatments: Two (grafting and rooted cuttings)

Replication: 3

Method for rooted cuttings: scions with 2 nodes were cut, dipped in rooting hormone and planted in soil

Results and Discussion



Seed yield from a donor plant after collection of possible scions for grafting / rooted cuttings

Additional seed yield from a donor plant from grafts / rooted cuttings

Increasing the success of survival of cuttings for clonal propagation or higher seed multiplication for determinate type beans is more critical compare to indeterminate type bean, where the growth of auxiliary shoots could be triggered for many cuttings. Grafting was a better tool compared to rooted cuttings for determinate type beans perpetuation and multiplication.

Methodology for All Crops

Method: Wedge grafting

Number of grafts – 10 per treatment / replication

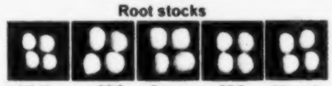
Lentil grafting

Treatments – 6
Replications - 4

Grafting of lentil on their rootstocks is very difficult as the stem girth is very thin which makes the grafting procedure difficult. Faba bean root stocks, which have large thick stems, were tried successfully with lentil scions on a preliminary basis. An experiment is in progress to identify the best faba rootstock for lentil grafting. Initial observations on the establishment of grafts (mean % survival of grafts) indicate that Taboar and Snowbird can be used as rootstocks with 65-70% success for Redberry scions and Taboar and Disco can be used for grafting Plato scions with 45-50% success.

Pea Grafting

Root stocks



Alfetta CDC Centennial Cooper CDC Golden Nitouche

Scions

1) *Pisum sativum* ssp *abyssinicum* 358616 (wild pea);
2) *P. sativum* ssp. *elatius* 560056 (wild pea);
3) Intervarietal F_1 (Tudor x CDC Striker);
4) *P. sativum* cultivar (CDC Rocket);
5) Self grafts

Results and Discussion

There were no significant effect of rootstock on any of the scions for mean % survival of grafts, seed yield and days to flowering. An average 75-80% survival was obtained through grafting. Any of the rootstocks above can be successfully used for grafting in cultivated and wild peas and in their interspecific hybrids for seed multiplication and perpetuation.

Faba Bean Grafting

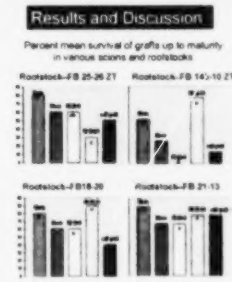
Rootstocks: FB 25-26 ZT; FB 143-10 ZT; FB 18-20 ; FB 21-13

Scions: Gloria ZT; Disco ZT; FB 20-5 ; FB 25-21

Total treatments: 16

Results and Discussion

Percent mean survival of grafts up to maturity in various scions and rootstocks



Significant differences for the % mean survival of scion grafts were observed for various root stocks (Fig). Rootstock FB 21-13 was found to be the best root stock with at least 60% mean survival of grafts for all the scions. Rootstock FB143-10 ZT was found to be least compatible for grafting including self grafts. All the rootstocks except FB 21-13 were found to be significantly less compatible for self grafting compared to grafting with other donor scions.

Acknowledgements

Saskatchewan pulse Growers

Saskatchewan Ministry of Agriculture

GRAFTING AS A TOOL FOR GENETIC ENHANCEMENT IN LEGUMES WITH A SPECIAL EMPHASIS ON INTERSPECIFIC BREEDING

Valarmathi Gurusamy, Kirstin Bett, Tom Warkentin and Albert Vandenberg

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Background and Objectives

Genetic improvement efforts for common bean, pea, faba bean and lentil include the introduction of genes from unadapted parents and wild species. Three common barriers in breeding strategies in these large seeded pulse crops are: (1) the low number of F_1 seeds produced per pollination, (2) the low seed increase ratio, and (3) the difficulty in rooting plants derived from doubled haploid culture (pea) or interspecific hybrids produced through *in vitro* techniques (bean / lentil). Seed quantity is generally limiting in early generation screening for multiple breeding objectives. Rooting through stem cuttings can be difficult for these crops, especially for interspecific hybrids between common bean and *P. acutifolius* and *P. angustissimus*, which are sources of genes for many useful traits (e.g., frost tolerance and disease resistance). Standardization of an efficient alternative asexual propagation technique will increase breeding efficiency through mass multiplication of early generation clones in these crops. Our objectives were to standardize grafting techniques and identify suitable root stocks for each crop so that these techniques can eventually become standard tools in breeding programs.

Materials – Common Bean

Root stocks

Scions

1) ***Phaseolus acutifolius*** **WI15578;**
2) ***P. angustissimus*** **PI535272;**
3) **Intervarietal F_1 of *P. vulgaris*;**
4) ***P. vulgaris*** **cultivar (CDC Whitecap);**
5) **Interspecies F_1** (*P. acutifolius* x *P. angustissimus*);
6) **Self grafts**

Methods – Common Bean

6-7 day old root stocks were cut below the cotyledons and 1-2 node scions were grafted onto them (Fig 2a). High humidity was maintained by covering plant + pot tightly with a polythene bag (Sigma Sun bags; Fig 3b). Bags were slowly opened and removed after observing growth of the scions by one additional node (Fig 3c). Observations on survival of grafts, days to flowering, total number of pods and seeds per graft were recorded on established grafts (Fig 3d, e & 2b, 2c). The experiment was conducted in controlled growth chambers with 2 replications.

Grafting in *Phaseolus* sp.

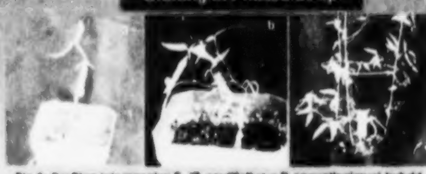


Fig 2: Grafting interspecies F_1 (*P. acutifolius* x *P. angustissimus*) hybrid scions onto *P. vulgaris* ICA Pijao root stocks

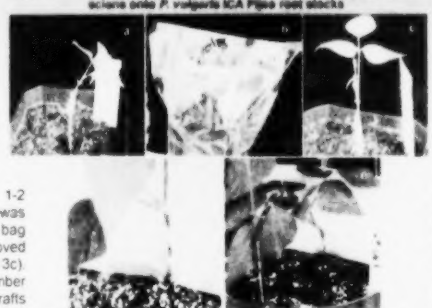


Fig 3 (a –e): Grafting steps - using *P. vulgaris* CDC Whitecap as root stock and interspecific F_1 scion (*P. vulgaris* NY5-161 x *P. acutifolius*)

Results and Discussion (Common Bean)

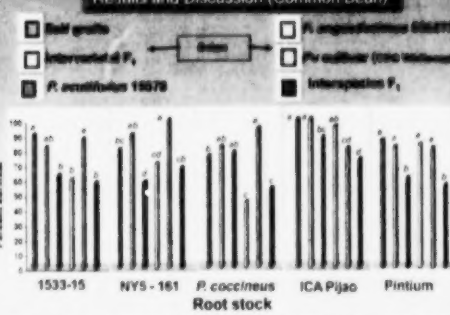


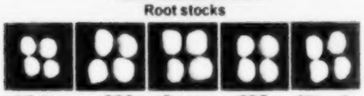
Fig 1. Percent survival of scions of different species on various root stocks of common bean

A significant influence of root stock (*P. vulgaris* and *P. coccineus*) and an interaction with scions of different species was observed for the percent survival of grafts only, **but not for seed yield or pod yield.** *P. vulgaris* ICA Pijao was the best root stock for survival of the scions of wild species, interspecies hybrids and inter- varietal hybrids upon grafting (Fig 1). Although *P. coccineus* was asexually compatible for grafting with *P. vulgaris* and *P. acutifolius* scions, it was comparatively less compatible with the scions of *P. angustissimus*. Thus, *P. vulgaris* ICA Pijao is recommended as a root stock in bean for improved early generation seed multiplication and interspecies breeding.

Grafting in *Pisum* Sp.

Materials - Pea

Root stocks



Scions

1) ***Pisum sativum* ssp *abyssinicum*** 358616 (wild),
2) ***P. sativum* ssp *elatius*** 560056 (wild),
3) **Intervarietal F_1** (Tudor x CDC Striker),
4) ***P. sativum* cultivar** (CDC Rocket),
5) **Self grafts**

Results and Discussion

A total of 25 combinations of grafts (5 root stocks and 5 scions) was produced as described for common bean, except that grafting onto the root stock occurred above the 2nd-3rd node (Fig a). There were no significant effect of root stock on any of the scions for mean % survival of grafts and seed yield. An average 75-80% survival was obtained through grafting. Any of the root stocks above can be successfully used for grafting in cultivated and wild peas. Compatibility of the *P. sativum* root stock with wild pea scions indicates the feasibility of grafting for interspecies breeding, double haploidy breeding, seed multiplication and for perpetuation.

Common Bean - Grafting vs Rooted cuttings

Materials: Scion - NY5-161 (Determinate type genotype),
Root stock – ICA Pijao

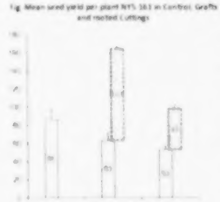
Treatments: 2 - grafting and rooted cuttings)

Replication: 3

Method for rooted cuttings: scions with 2 nodes were cut, dipped in rooting hormone and planted in soil

Results and Discussion

Fig. Mean seed yield per plant NY5 161 in Control, Grafts and rooted Cuttings



Seed yield from a donor plant after collection of possible scions for grafting / rooted cuttings

Additional seed yield from a donor plant from grafts / rooted cuttings

Increasing the success of survival of cuttings for clonal propagation or higher seed multiplication for determinate type beans is more critical compare to indeterminate type bean, where the growth of auxiliary shoots could be triggered for many cuttings. Grafting was a better tool compared to rooted cuttings for perpetuation and multiplication of common beans with determinate growth habit.

Faba Bean Grafting

Rootstocks: FB 25-26 ZT, FB 143-10 ZT, FB 18-20, FB 21-13

Scions: Gloria ZT, Disco ZT, FB 20-5, FB 25-21

Total treatments: 16

Results and Discussion

Significant differences for the % mean survival of scion grafts were observed for faba bean root stocks. Root stock FB 21-13 was found to be the best root stock with at least 60% mean survival of grafts for all the scions. Root stock FB143-10 ZT was found to be least compatible for grafting including self grafts. All the root stocks except FB 21-13 were found to be significantly less compatible for self grafting compared to grafting with other donor scions. Grafting can be successfully used in faba bean breeding to overcome the rooting problems with stem cuttings.

Lentil Grafting

Grafting of lentil on their root stocks is very difficult because the stems are very thin. Faba bean root stocks, which have large thick stems, were tried successfully with lentil scions on a preliminary basis. An experiment is in progress to identify the best faba bean rootstock genotype for lentil grafting.

Acknowledgements

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Materials – Common Bean

Scions

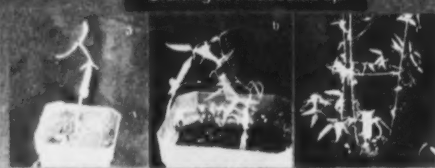
1) *Phaseolus acutifolius* W615576;
2) *P. angustissimus* PI535272;
3) Intervarietal F_1 of *P. vulgaris*;
4) *P. vulgaris* cultivar (CDC Whitecap);
5) Interspecies F_1 (*P. acutifolius* x *P. angustissimus*);
6) Self grafts.

Methods – Common Bean

[illegible]

6-7 day old root stocks were cut below the cotyledons and 1-2 node scions were grafted onto them (Fig 2a). High humidity was maintained by covering plant + pot tightly with a polythene bag (Sigma Sun bags; Fig 3b). Bags were slowly opened and removed after observing growth of the scions by one additional node (Fig 3c). Observations on survival of grafts, days to flowering, total number of pods and seeds per graft were recorded on established grafts (Fig 3d, e & 2b, 2c). The experiment was conducted in controlled growth chambers with 2 replications.

Grafting in *Phaseolus* sp.



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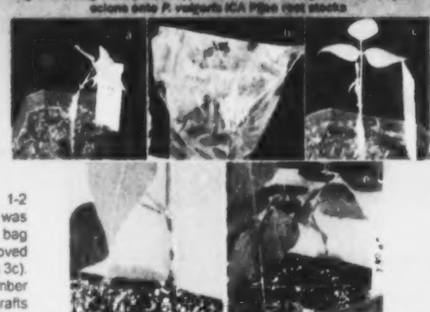

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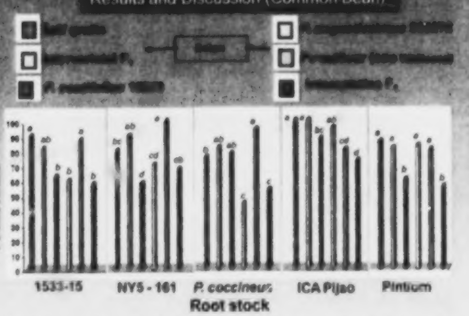
Results and Discussion (Common Bean)



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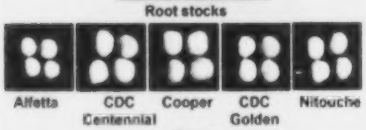
Grafting in *Pisum* Sp

Materials - Pea

Root stocks



Scions

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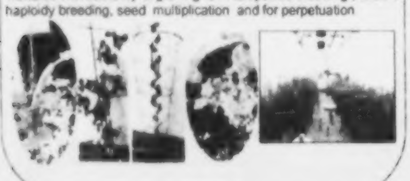

Common Bean - Grafting vs Rooted cuttings

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Treatments: 2 - grafting and rooted cuttings)

Replication: 3

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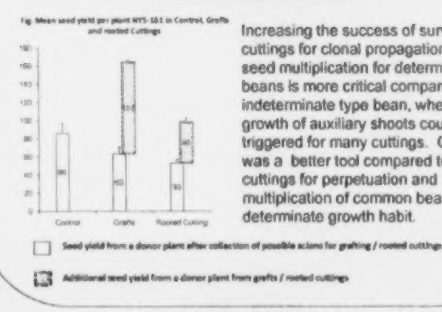
Results and Discussion



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